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Increased alveolar nitric oxide concentration is related to nocturnal oxygen desaturation in obstructive sleep apnoea

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ABSTRACT

Purpose: To assess distal/alveolar inflammation in patients with suggestive symptoms of obstructive sleep apnoea (OSA) using exhaled nitric oxide (NO) measured by two-compartment model (2-CM) after correction for axial NO back-diffusion (trumpet model).
Methods: Ninety five patients suspected for OSA prospectively underwent pulmonary function test, overnight polysomnography (PSG), and exhaled NO measurement. Patients with apnoea–hypopnoea index (AHI) < 5/hour were included in non-OSA group. Exhaled NO was repeatedly measured after PSG in 21 OSA patients and 8 non-OSA subjects.
Results: Alveolar NO concentration (C_{ANO}) was significantly higher in OSA patients (n = 71; 4.07 ± 1.7ppb) as compared with non-OSA subjects (n = 24; 2.24 ± 1.06ppb; $p < 0.0001$) whilst maximal bronchial NO flux (J'_{awNO}) and fractional exhaled NO (F_{ENO}) did not differ between the two groups. C_{ANO} was strongly associated to AHI ($r = 0.701$; $p < 0.0001$) and to recording time with $SaO_2 < 90\%$ (ST-90%; $r = 0.659$; $p < 0.0001$) in OSA patients but not in non-OSA persons. The area under ROC curve for screening patients with OSA and significant nocturnal oxygen desaturation (ST-90% > 1%) was 0.865 ± 0.036 (95% IC, 0.793–0.937; $p < 0.0001$). C_{ANO} at 4.5 ppb could detect these patients with specificity of 94% and sensitivity of 46%. Increase of C_{ANO} measured after PSG was significantly related to oxygen desaturation index (ST-90%) in OSA patients.
Conclusions: Increased alveolar NO concentration was related to the severity of nocturnal oxygen desaturation in patients with OSA, linking the distal airway inflammation to intermittent hypoxia. (250 words)

1. Introduction

Obstructive sleep apnoea (OSA) is an independent risk factor for cardiovascular morbidity and mortality [1], due to increased production of reactive oxygen species and pro-inflammatory cytokines, resulting from chronic intermittent hypoxia–reoxygenation [2]. Oxidative stress and inflammation cause endothelial dysfunction leading to cardiovascular diseases [3].Thus, evaluation of pulmonary inflammation might be useful to screen for OSA and to predict its severity.

Nitric oxide (NO) plays an important role both as a physiological modulator of vascular tone and as a pathological pro-inflammatory biomarker implicated in many lung disorders [4]. NO can be easily measured in the exhaled air, and there are theoretical grounds to hypothesise that concentration of exhaled NO (F_{ENO}) may change in the two principal pathological processes observed in OSA: pulmonary inflammation and endothelial dysfunction. Increased exhaled NO reflects lung inflammation by over-expression of the inducible NO synthase (NOS) as observed in asthma and systemic sclerosis (SSc) [5,6], whilst reduced exhaled NO levels can be found in cardiovascular disorders associated with endothelial dysfunction such as pulmonary hypertension [7] and chronic heart failure [8], due to decreased endothelial NOS expression and activity.

In patients with OSA, lung inflammation and vascular injuries usually co-exist with, however, different degrees of severity. F_{ENO}, which reflects NO production from the large airways, has been found either

Abbreviations: 2-CM, two-compartment model; AHI, apnoea–hypopnoea index; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; C_{ANO}, alveolar nitric oxide concentration; F_{ENO}, fractional exhaled nitric oxide; J'_{awNO}, maximal bronchial nitric oxide flux; NOD, nocturnal oxygen desaturation; OSA, obstructive sleep apnoea; PSG, polysomnography; ROC, receiver operating characteristic; Se, sensitivity; Sp, specificity; ST-90%, percentage of recording time with $SaO_2 < 90\%$ on total sleep time.

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unchanged [9] or increased [10–17] in patients with OSA. F_{ENO} is however a poor marker of NO production in the distal parts of the lungs, i.e. small airways and alveolar spaces. The two-compartment model (2-CM) allows quantification of maximal bronchial NO flux (J'_{awNO}) and steady-state alveolar NO concentration (C_{ANO}) [18]. Using this simplified model, two studies reported a decrease of C_{ANO} in patients with OSA [10,15] suggesting endothelial dysfunction that might be linked to systemic hypertension [10]. We hypothesised that in patients with advanced OSA and vascular diseases, distal/alveolar NO production might decrease [10,15] but in patients with moderate OSA and associated lung inflammation, C_{ANO} might increase as observed in patients with systemic sclerosis [6].

It is recently suggested that taking into account NO axial back-diffusion, related to the trumpet shape of the cross-sectional area of the tracheal tree [18], can better characterise the proximal and distal exhaled NO origins in healthy subjects [19] and SSc patients [20].

In this prospective study, we aimed to assess the distal/alveolar inflammation in patients with suggestive symptoms of OSA, using this novel approach. We also studied the variation of exhaled NO after overnight PSG recording in OSA and non-OSA patients to see whether this variation was associated with sleep apnoea parameters.

2. Methods

2.1. Study population

All subjects (≥18 years-old) were recruited from our Sleep Research Unit, Department of Physiology, Cochin Hospital, Paris, France. They were consecutively referred for OSA diagnosis with suggestive symptoms (American Association of Sleep Medicine, AASM criteria) during a period of 2 years, from January 1, 2009 to December 31, 2010 [21]. The study was approved by the Ethics Committee of our institution and informed consents were obtained from all participants.

We excluded patients with advanced cardiovascular diseases or respiratory disorders, current smokers, patients with upper or lower respiratory infections, and those receiving oral corticosteroids within the last 4 weeks since these factors could modify exhaled NO.

All patients underwent thorough physical examination with medical history and pulmonary function tests before inclusion. On the examination day, exhaled NO was measured in the afternoon, then patients were submitted to overnight in-laboratory polysomnography (PSG). On the next morning, exhaled NO was re-evaluated in 21 patients with OSA (defined as AHI ≥5/hour) and 8 patients without OSA. We included 30 healthy non-smokers (mainly from our medical staff and students) having exhaled NO measurement as controls.

2.2. Lung function measurement

Pulmonary function tests (PFT), assessing forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and total lung capacity (TLC) (MasterScreen® Body; VIASYS Healthcare GmbH, Hoechberg, Germany), were performed according to the international recommendations [22]. Blood gas analysis was done on the same day.

2.3. Exhaled NO measurement

Exhaled NO was measured using a chemiluminescent NO analyser (EndoNO 8000®; SERES, Aix-en-Provence, France), according to ATS/ERS recommendations [23]. NO analyser was calibrated daily with a standard NO source (100 ppb, Air Liquid, Paris, France). Exhaled NO measurement was strictly performed in all subjects during the medical visit preceding the PSG recordings, as previously described [20,24]. Patients who cannot perform a regular exhalation of more than 8 seconds to obtain a steady-state alveolar NO concentration during 3 seconds were excluded from the study [24].

Correction of J'_{awNO} and C_{ANO} using the trumpet (TP) model to take into account axial NO back-diffusion was then applied for all measurements [19]. We used the linear relationship to express the variation of V'_{NO} (pl/s: picolitre/second) as a function of V'_E (ranging from 100 to 250 ml/s):

$$V'_{NO} = \left(C_{ANO(TP)} + J'_{awNO(TP)} \cdot 0.00078\right) \cdot V'_E + J'_{awNO(TP)}/1.7$$

The slope of this linear relationship S and the intercept I was obtained by plotting the V'_{NO} against V'_E, allowing the calculation of $C_{ANO(TP)}$ and $J'_{awNO(TP)}$ from the $C_{ANO(2CM)}$ and $J'_{awNO(2CM)}$ as previously described [18,20].

2.4. Polysomnography

All patients underwent overnight polysomnography (PSG) using a Medcare data-acquisition system (Rembrandt Analysis Manager, Buffalo, NY, USA) with standard electrodes and sensors according to AASM recommendations [25]. Briefly, electroencephalography electrodes applied at A2-C4, C4-C3, C3-A1, and C3-O1, two electro-oculography, submental and anterior tibial electromyography channels were recorded. For respiratory parameters, thoracic and abdominal movements by inductance plethysmography, thermistors and nasal pressure cannulas were used simultaneously. Arterial oxygen saturation (SaO_2) was measured using pulse oximeter. To establish sleep stages, recorded nocturnal PSG were visually scored on the basis of 30-second epochs, using Rechtschaffen and Kales criteria, by two independent medical doctors with more than 10 year-experience in sleep medicine [26]. Apnoea was defined as a complete cessation of oro-nasal airflow ≥10 seconds. Hypopnoea was defined as an important reduction in airflow (≥50%) for ≥10 seconds or moderate reduction (<50%) associated with EEG arousals and/or oxygen desaturation (SaO_2 decrease ≥3%). The apnoea–hypopnoea index (AHI) was defined as the addition of apnoea episodes and hypopnoea episodes per hour during sleep. Patients with OSA (AHI ≥5) were divided into mild-to-moderate group (5 ≤ AHI <30) and severe one (AHI ≥30) [26]. Subjects with AHI <5 constituted the non-OSA group. For the nocturnal oxygen desaturation (NOD), we recorded the mean SaO_2, nadir SaO_2 and the percentage of recording time with SaO_2 <90% on total sleep time (ST-90%). Patients with significant NOD were defined as ST-90% of more than 1% [27].

2.5. Statistical analysis

Data were analysed using SPSS 16.0 (Chicago, IL, USA). Values were expressed as mean ± standard derivation (SD) for continuous parameters, number and percentage for categorical variables. Normal distribution was determined using Kolmogorov–Smirnov test. Comparisons were made by Student's *t*-test or Mann–Whitney test for quantitative variables and Chi-square test or Fisher's exact test for qualitative variables, as appropriate. Exhaled NO levels before and after PSG were compared using Wilcoxon matched-pairs rank test. Linear correlations between C_{ANO} with AHI and ST-90% were done using Spearman's method. A multivariate linear regression was used to examine the association of AHI with C_{ANO} and BMI. Statistical significance was two-sided at $p < 0.05$.

Diagnostic performance of C_{ANO} for detecting patients with OSA (AHI ≥5/hour) and significant NOD (ST-90% > 1%) was done by the ROC curve. Overall discriminatory ability of ROC curve was determined by the area under ROC curve (mean ± SD; [95% CI]).

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Table 1
Anthropometric, respiratory characteristics and exhaled nitric oxide parameters.

	OSA patients (n = 71)	Non-OSA patients (n = 24)	p
Age (years)	58.2 ± 10.3	52.6 ± 15.3	0.1
Male/female (n)	46/25	11/13	0.15
Ex-smoking (n; %)	10; 14.1	2; 8.3	0.72
Alcohol user (n; %)	12; 16.9	3; 16.7	0.75
Epworth scale score	7.5 ± 5.2	7.3 ± 4.1	0.85
BMI (kg/m^2)	29.9 ± 6.5	27.4 ± 5.7	0.09
Obesity (n; %)	30; 42.3	9; 37.5	0.68
Systemic hypertension (n; %)	31; 43.7	6; 25	0.1
Arrhythmias (n; %)	6; 8.4	0; 0	0.33
Coronary arterial disease (n; %)	1; 1.4	0; 0	1
Type 2 diabetes (n; %)	16; 22.5	2; 8.3	0.15
FEV_1 (% predicted)	101.9 ± 14.2	101.1 ± 15.2	0.87
FVC (% predicted)	102.4 ± 15.4	106.9 ± 13.5	0.38
FEV_1/FVC	77.9 ± 6.8	76.5 ± 5.1	0.52
TLC (% predicted)	106.9 ± 15.9	106.9 ± 11.8	0.99
PaO_2 (mmHg)	88.6 ± 7.1	84.8 ± 6.5	0.32
$PaCO_2$ (mmHg)	37.8 ± 3.5	41 ± 4.1	0.12
AHI events/hour of sleep	26.4 ± 17.4	2.5 ± 1.5	**<0.0001**
Diurnal SaO_2 (%)	95.8 ± 1.6	96.5 ± 1.5	0.06
Nocturnal SaO_2 (%)	93.8 ± 1.5	94.8 ± 1	**0.001**
Nadir SaO_2 (%)	84.1 ± 8.2	92.3 ± 3.8	**<0.0001**
Recording time SaO_2 < 90% (%)	7.3 ± 10.3	0.5 ± 0.8	**<0.0001**
C_{ANO} (ppb)	4.07 ± 1.70	2.24 ± 1.06	**<0.0001**
J'_{awNO} (pl/s)	1042 ± 948	1044 ± 563	0.99
$F_{ENO.50}$ (ppb)	17.2 ± 11.5	16.7 ± 14.2	0.79
R^2	0.981 ± 0.021	0.975 ± 0.022	0.13

Data were expressed as mean ± SD. OSA: obstructive sleep apnoea; BMI: body mass index; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity; AHI: apnoea–hypopnoea index; C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values.

3. Results

We prospectively included 95 subjects (57 men) with mean age (56.8 ± 11.9 years) from January 2009 to December 2010. Characteristics of patients with and without OSA were presented in Table 1. There was no significant difference in term of age, sex, ex-smoking and alcohol status, percentage of obesity, and daytime sleepiness between the two groups. There was a tendency of increased overweight severity in the OSA group (p = 0.09). Percentages of type 2 diabetes and cardiac comorbidities including arterial hypertension, arrhythmias, and coronary arterial disease were not significantly different between OSA patients and non-OSA subjects (p > 0.05). All patients had normal results in pulmonary function test and arterial blood gas.

Table 2
Exhaled nitric oxide parameters in patients with mild to moderate OSA compared with those with severe OSA.

	Mild-moderate OSA (5 ≤ AHI <30), n = 50	Severe OSA (AHI ≥30), n = 21	p
C_{ANO} (ppb)	3.46 ± 1.36	5.5 ± 1.56	**<0.0001**
J'_{awNO} (pl/s)	1074 ± 942	966 ± 980	0.65
$F_{ENO.50}$ (ppb)	16.9 ± 11.4	17.6 ± 12.0	0.86

Data were expressed as mean ± SD, unless otherwise noted. OSA: obstructive sleep apnoea; AHI: apnoea–hypopnoea index; C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values.

Regarding respiratory parameters, OSA patients had significantly higher AHI (26.4 ± 17.4 events/hour versus 2.5 ± 1.5 events/hour, p < 0.0001), lower mean nocturnal SaO_2 (93.1 ± 1.5% versus 94.6 ± 1.9%, p = 0.001), lower nadir SaO_2 (84.1 ± 8.2% versus 92.3 ± 3.8%, p < 0.0001), and longer recording time with SaO_2 < 90% (7.3 ± 10.3% versus 0.5 ± 0.8%, p < 0.0001) as compared with non-OSA subjects.

3.1. Exhaled nitric oxide parameters and OSA severity

Mean level of C_{ANO} from patients with OSA (n = 71; 4.07 ± 1.7 ppb) was significantly higher than that from non-OSA subjects (n = 24; 2.24 ± 1.06 ppb; p < 0.0001). However, there was no significant difference in J'_{awNO} and $F_{ENO.50}$ between these two groups (p > 0.05) (Table 1). We also measured exhaled nitric oxide in 30 healthy, non-smoking, and non-obese subjects and found no significant difference in C_{ANO} (1.9 ± 1.16 ppb; p = 0.37), J'_{awNO} (1149 ± 539 pl/s; p = 0.48), and $F_{ENO.50}$ (16.3 ± 6.6 ppb; p = 0.86) as compared with non-SAS subjects.

Regarding the severity of OSA, patients with severe OSA (AHI ≥ 30/hour) had a significantly higher level of C_{ANO} (n = 21; 5.5 ± 1.56 ppb) as compared with those with mild-to-moderate OSA (n = 50; 3.46 ± 1.36 ppb; p < 0.0001). There was no difference in J'_{awNO} and $F_{ENO.50}$ between these two groups (p > 0.05) (Table 2). Interestingly, we found a positive linear correlation between C_{ANO} and AHI (n = 71; r = 0.701; p < 0.0001) in patients with OSA while no correlation was found in the non-OSA group (p = 0.18) (Fig. 1).

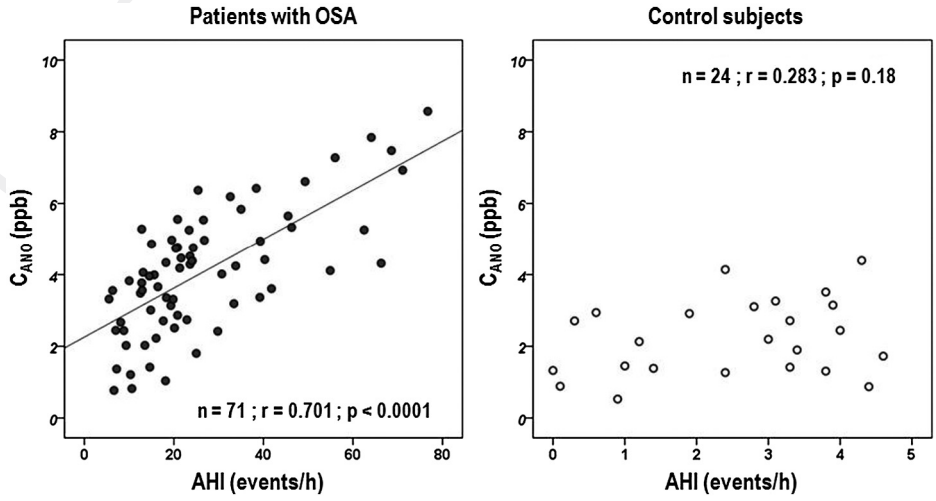

Fig. 1. Relation between alveolar concentration of exhaled nitric oxide (C_{ANO}) and apnoea–hypopnoea index (AHI) in 71 patients with OSA and in 24 non-OSA subjects. Exhaled nitric oxide (NO) was measured before polysomnography (PSG) by using the two-compartment model with correction for axial NO diffusion and PSG was recorded as described in Methods. C_{ANO} was significantly associated to AHI in OSA patients (p < 0.0001) but not in non-OSA persons (p = 0.18).

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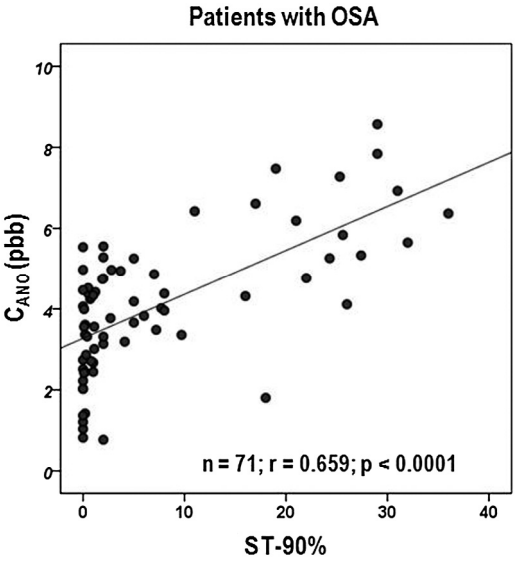

Fig. 2. Relation between alveolar concentration of exhaled nitric oxide (C_{ANO}) and percentage of recording time with $SaO_2 < 90\%$ on total sleep time (ST-90%) in 71 patients with OSA (apnoea–hypopnoea index (AHI) ≥ 5 events/hour). Exhaled nitric oxide (NO) was measured before polysomnography (PSG) by using the two-compartment model with correction for axial NO diffusion and PSG was recorded as described in Section 2. C_{ANO} was significantly linked to ST-90% in OSA patients ($p < 0.0001$).

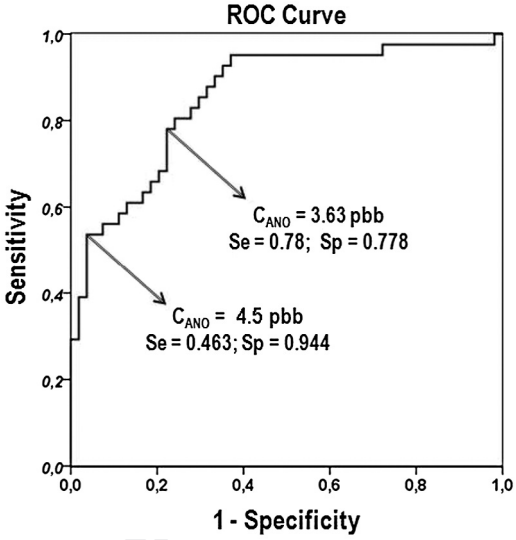

Fig. 3. Receiver operating characteristic (ROC) curve of predicting the patients with OSA (AHI ≥ 5/h) and significant nocturnal oxygen desaturation (recording time with $SaO_2 < 90\%$ more than 1% TST) among the whole study population (n = 95). Area under ROC curve: 0.865 ± 0.036 ($p < 0.0001$; 95% CI [0.793–0.937]). Se: Sensitivity; Sp: Specificity; C_{ANO}: alveolar nitric oxide concentration by "Trumpet" model.

3.2. Exhaled nitric oxide parameters and nocturnal oxygen desaturation (NOD)

Patients with OSA were then divided into a group with NOD (ST-90% > 1% TST), and the other without NOD. Mean level of C_{ANO} from patients with NOD (n = 41; 4.81 ± 1.6 ppb) was significantly higher than that from patients without NOD (n = 30; 3.05 ± 1.25 ppb; $p < 0.0001$). There was no difference in J'_{awNO} and $F_{ENO.50}$ between these two groups ($p > 0.05$) (Table 3). We also showed a significant linear correlation between C_{ANO} and ST-90% in OSA patients (n = 71; r = 0.659; $p < 0.0001$) (Fig. 2).

Table 3
Exhaled nitric oxide parameters and nocturnal oxygen desaturation (NOD)* in OSA patients.

	Patients without NOD* (n = 30)	Patients with NOD* (n = 41)	p
C_{ANO} (ppb)	3.05 ± 1.25	4.81 ± 1.6	**<0.0001**
J'_{awNO} (pl/s)	1077 ± 1064	1017 ± 866	0.79
$F_{ENO.50}$ (ppb)	16.6 ± 13.0	17.6 ± 10.4	0.24

*Nocturnal oxygen desaturation was defined as sleeping time with $SaO_2 < 90\%$ more than 1% of total sleep time (# 5 minutes). Data were expressed as mean ± SD. OSA: obstructive sleep apnoea; AHI: apnoea–hypopnoea index; C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values.

3.3. Alveolar exhaled nitric oxide for detecting OSA patients with NOD

Because the levels of C_{ANO} were highly correlated to the severity of OSA as measured by AHI and to the NOD degree as quantified by ST-90%, we used it for screening this group of patients at highest cardiovascular risk. The whole population was then divided into 2 groups: 41 patients with OSA (AHI ≥ 5) and NOD (ST-90% > 1%), and 54 patients without OSA (AHI < 5) and/or without NOD (ST-90% ≤ 1%). The area under ROC curve was statistically significant ($p < 0.0001$; AUROC: 0.865; [0.793–0.937]) (Fig. 3). The highest Youden index was at C_{ANO} level of 3.63 ppb with sensitivity of 78% and specificity of 77.8%. Another threshold of C_{ANO} at 4.5 ppb yielded higher specificity of 94.4% but lower sensitivity of 46.3%.

3.4. Variation of exhaled nitric oxide after overnight polysomnography (PSG)

In 21 patients with OSA, median value of C_{ANO} measured after overnight PSG (6.27 ppb; [4.11–8.3]) was significantly higher than before PSG (4.06 ppb; [3.42–5.15]; $p = 0.0001$). In non-OSA subjects, there was no significant difference between C_{ANO} before PSG (n = 8; 2.02 ppb; [1.43–2.86]) and those recorded after PSG (2.63 ppb; [2.06–3.73]; $p = 0.12$) (Table 4 and Fig. 4).

C_{ANO} variation (after PSG C_{ANO} – before PSG C_{ANO}) was significantly associated to ST-90% (n = 21; r = 0.595; $p = 0.004$) (Fig. 5) but not to AHI ($p = 0.88$). There was no difference in median levels of

Table 4
Increased exhaled nitric oxide parameters measured before and after in-laboratory polysomnography (PSG) in patients with OSA and non-OSA subjects.

	Patients with OSA (n = 21)			Non-OSA subjects (n = 8)		
	Before PSG	After PSG	p	Before PSG	After PSG	p
C_{ANO} (ppb)	4.06 (3.42–5.15)	6.27 (4.11–8.3)	**0.0001**	2.02 (1.43–2.86)	2.63 (2.06–3.73)	0.12
J'_{awNO} (pl/s)	885 (573–1356)	878 (577–1140)	0.75	732 (498–1055)	918 (499–1250)	0.48
$F_{ENO.50}$ (ppb)	14.7 (10.6–22.4)	16.8 (11.5–21.9)	0.15	12.5 (8.7–16.9)	14.1 (9.0–16.8)	0.67

Data were expressed as median (IQT) and comparisons were made by Wilcoxon matched-pairs signed-rank test; OSA: Obstructive Sleep Apnoea; PSG: overnight inpatient polysomnography; C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values (<0.05).

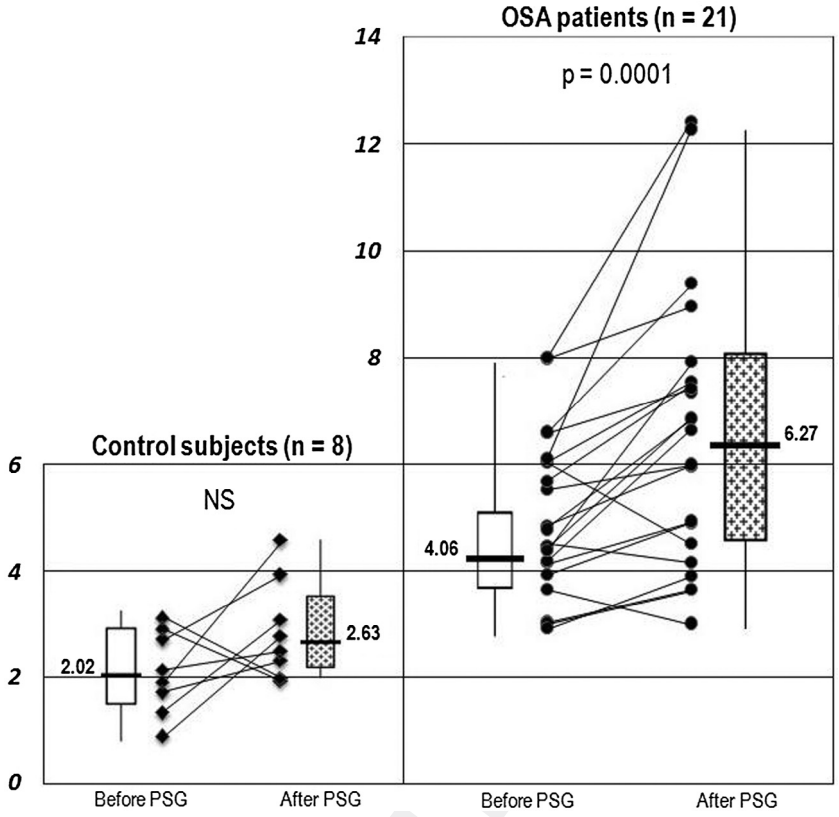

Fig. 4. Increase of alveolar exhaled nitric oxide (C_{ANO}) after one night sleep with in-laboratory polysomnography (PSG) recording. Exhaled nitric oxide (NO) was measured after polysomnography (PSG) in 21 patients with OSA and in 8 non-OSA subjects. C_{ANO} levels post-sleep were significantly increased ($p = 0.0001$) to approximately 150% of pre-sleep levels only in OSA patients.

J'_{awNO} and $F_{ENO.50}$ before and after PSG in OSA patients or in non-OSA subjects ($p > 0.05$) (Table 4).

3.5. Exhaled nitric oxide parameters with age, obesity, and systemic hypertension

There was no significant correlation between C_{ANO}, J'_{awNO} or $F_{ENO.50}$ with age ($p > 0.05$) in patients with OSA and in non-OSA subjects (data not shown).

In OSA patients, there was a significant positive relation between C_{ANO} and BMI ($n = 71$; $r = 0.247$; $p = 0.038$). However, in linear regression analysis, AHI was significantly associated to C_{ANO} (β: 8.433 ± 0.865; $p < 0.0001$) but not to BMI (β: 0.288 ± 0.203; $p = 0.16$). There was no association between J'_{awNO} or $F_{ENO.50}$ and BMI (Table 5).

C_{ANO} was not significantly different ($p > 0.05$) between OSA patients with and without hypertension. However, in the non-OSA group, C_{ANO} was significantly decreased in those with hypertension ($n = 6$; 1.29 ppb; [0.88–1.39]) as compared with non-hypertensive subjects ($n = 18$; 2.71 ppb; [1.85–3.17]; $p = 0.002$) (Table 6).

Table 5
Correlation between exhaled nitric oxide parameters and the severity of obesity assessed by BMI in patients with OSA and non-OSA subjects.

	Patients with OSA (n = 71)		Non-OSA subjects (n = 24)	
	r	p	r	p
C_{ANO} (ppb)	0.247	**0.038**	–0.496	**0.014**
J'_{awNO} (pl/s)	–0.074	0.54	0.329	0.12
$F_{ENO.50}$ (ppb)	–0.041	0.74	0.237	0.26

Correlations between BMI and NO parameters were made by Pearson's method in OSA patients and by Spearman's in control subjects. BMI: body mass index; OSA: obstructive sleep apnoea; C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values (<0.05).

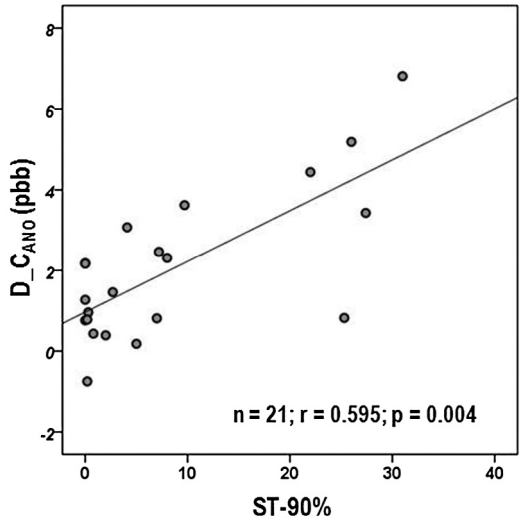

Fig. 5. Relation between increased levels of alveolar exhaled nitric oxide (D_C_{ANO}) after sleep and percentage of recording time spent at a SaO_2 <90% (ST-90%) in 21 patients with OSA. Increase of C_{ANO} post-sleep was significantly associated ($p = 0.004$) to nocturnal oxygen desaturation index as evaluated by ST-90%.

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Table 6
Exhaled nitric oxide parameters in patients with OSA and non-OSA subjects according to systemic arterial hypertension (SHT).

	Patients with OSA			Non-OSA subjects		
	No SHT	SHT	p	No SHT	SHT	p
Number	40	31		18	6	
C_{ANO} (ppb)	3.92 ± 1.81	4.26 ± 1.54	0.29	2.71 (1.85–3.17)	1.29 (0.88–1.39)	**0.002**
J'_{awNO} (pl/s)	1003 ± 1048	1094 ± 815	0.69	820 (506–1202)	1219 (1075–2150)	**0.023**
$F_{ENO.50}$ (ppb)	16.5 ± 12.8	18 ± 9.6	0.59	12.7 (9.6–16.8)	16.4 (14.9–28)	**0.046**

OSA: obstructive sleep apnoea C_{ANO}: alveolar nitric oxide concentration; J'_{awNO}: maximal bronchial flux of nitric oxide; $F_{ENO.50}$: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values (<0.05).

4. Discussion

In this prospective study, we found that C_{ANO} was significantly increased in patients with OSA as compared with non-OSA subjects. Furthermore, C_{ANO} was correlated to the severity of OSA and the degree of nocturnal oxygen desaturation (NOD). The result was reinforced by the positive linear relationship between overnight C_{ANO} variation and the NOD degree as assessed by the percentage of recording time spent at a $SaO_2 < 90\%$ (ST-90%). In addition, this correlation was only found in OSA patients. We finally proposed the threshold of C_{ANO} at 4.5 ppb, using the trumpet model, as the most relevant cut-off level to specifically detect patients with high risk of OSA and NOD among those with suggestive symptoms of OSA. These results were similar (data not shown) when we calculated exhaled NO parameters using the two-compartment model yielding C_{ANO} values slightly higher than those calculated from the trumpet model, as previously described [20].

Both inflammation and endothelial dysfunction are important features of OSA [2–4]. Several studies demonstrated increased exhaled NO, reflecting airway inflammation in patients with OSA. Upper airway inflammation has firstly been evidenced by an increased nasal NO [11,13] that was linked to over-expression of inducible NOS and 3-nitrotyrosine (3-NT) in palatine tonsils from patients with OSA as compared with healthy controls. High F_{ENO} levels were significantly correlated to the severity of OSA and to increased inducible NOS expression in induced sputum monocytes from OSA patients [12]. Thus, these inflammatory markers are tightly associated to the severity of OSA and, therefore, could be used in clinical monitoring of this syndrome. More recent studies [13,17] found a significant increase of F_{ENO} in OSA patients, which was partially reversible after 1-month CPAP treatment. These findings confirmed airway inflammation in patients with OSA (Table 7) that correlated to disease severity.

Several animal models that mimic OSA in humans have been used to study molecular and biological processes. In animals exposed to chronic intermittent hypoxia (CIH), increased expression of NF-kB and iNOS has been consistently found in the heart, the aorta [28], and in the hypoxia-sensitive carotid body [29], responsible for high 3-nitrotyrosine accumulation in these tissues. Jelic et al. [30] examined circulating endothelial progenitor cells and found simultaneous increased expression of iNOS and 3-nitrotyrosine, an oxidative and nitrosative stress that was reversible after efficient CPAP treatment [31]. These data support a critical role for iNOS in the development of the CIH-induced oxidative stress and

Table 7
Recapitulation of published data in exhaled nitric oxide measurements in patients with OSA.

	n	NO parameter	Method	Expiratory flow rates (mL/s)	Main results	Interpretation	Ref.
Olopade CO et al., Chest1997	20	Nasal NO (nNO) Oral NO (oNO)	Offline	NC	Increased nNO and oNO after sleep as compared with pre-sleep values in OSA patients but not in controls	Upper airway inflammation after sleep	[12]
Agusti AG et al., Sleep 1999	24	F_{ENO}	Online	NC	Unchanged F_{ENO} in OSA patients		[10]
Petrosyan M et al., Sleep Breath 2008	26	Nasal NO (nNO) F_{ENO}	Online	250	Increased nNO and F_{ENO} in OSA patients, partially reversed after 1-month CPAP therapy No difference of nNO and F_{ENO} between obese and non-obese controls	(Upper and lower) Airway inflammation and oxidative stress in OSA patients	[14]
Carpagnano GE et al., TranslRes2008	30	F_{ENO}	Online	45	Increased F_{ENO} in obese patients with or without OSA versus healthy controls	Lower airway inflammation in obese patients	[17]
Depalo A et al., J Intern Med 2008	18	F_{ENO} (NIOX)	Online	45	Increased F_{ENO} in OSA patients and obese patients versus healthy subjects Increased F_{ENO} correlated to percentage and inducible NOS of neutrophils in induced sputum	Airway inflammation in OSA and obese patients	[13]
Verhulst SL et al., Chest 2008	11	F_{ENO} (NIOX)	Online	50	Increased F_{ENO} in obese patients with habitual snoring or OSA versus normal weight or obese healthy subjects	Airway inflammation in snoring and OSA patients but not in obese controls	[15]
Chua AP et al., J Clin Sleep Med 2013	75	F_{ENO} (NIOX)	Online	50	Increased F_{ENO} in OSA patients, FENO correlates to severity and decreases after positive pressure therapy	Role of upper airway inflammation in the pathophysiology and treatment of OSA	[18]
Foresi A et al., Chest 2007	34	F_{ENO} and C_{ANO}	2 CM model	50, 120, 190, 250, and 300	Decreased C_{ANO} in OSA patients, and in OSA patients with hypertension (HT) versus those without HT	Linked to systemic hypertension	[11]
Fortuna AM et al., Respir Med 2011	30	F_{ENO} and C_{ANO}	2 CM model	10, 30, 100, and 200	Increased F_{ENO} and decreased C_{ANO} in OSA patients, restored after 3-month CPAP treatment	Airway inflammation and endothelial dysfunction in severe OSA patients	[16]

NO: nitric oxide; OSA: obstructive sleep apnoea; AHI: apnoea–hypopnoea index; C_{ANO}: alveolar nitric oxide concentration; F_{ENO}: fractional exhaled nitric oxide; NOS: nitric oxide synthase; CPAP: continuous positive airway pressure.

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inflammatory responses in OSA [2,3]. In another rat model, recurrent obstructive apnoeas alone or hypoxia/normoxia episodes alone could induce an inflammatory process by activating NF-kB and increasing TNF-α and IL-1β production in the lungs [32]. High levels of pro-inflammatory cytokines can stimulate the NF-kB pathway which, in turn, induced iNOS gene expression and NO overproduction [33]. These findings in animals were consistent with the main result of our study in humans that in OSA patients, the peripheral lung inflammation as assessed by C_{ANO}, was strongly related to the severity of OSA evaluated by apnoea–hypopnoea index (AHI) and nocturnal oxygen desaturation level (ST-90%).

Two studies using 2-CM model for exhaled NO measurement, however, found a decreased C_{ANO} in OSA patients [11,16]. Regarding demographic characteristics, their populations had more severe OSA and obesity as assessed by AHI and BMI, respectively, when compared with our patients. Percentage of OSA patients with systemic hypertension was also higher than that from our study [10]. These discrepancies might be linked to a peripheral/distal obstructive phenomenon, or to a decrease in NO synthesis and bioavailability related to endothelial dysfunction and advanced oxidative stress environment [2–4].

Our study showed that patients with OSA increased the levels of C_{ANO} after overnight PSG. This augmentation was correlated to the oxygen desaturation severity, linking alveolar inflammation to acute intermittent hypoxia–reoxygenation. To the best of our knowledge, this phenomenon was not yet explored using the same model of exhaled NO measurement. In 1997, Olopade et al. [11] found an increased nasal NO levels after sleep in OSA patients as compared with control subjects, confirming an enhanced upper airway inflammation by intermittent sleep apnoeas. Recently, Chua et al. [17] showed that post-sleep F_{ENO} levels were significantly higher than pre-sleep values and the rising levels of F_{ENO} correlated to the severity of OSA.

Increased C_{ANO} in OSA patients reflected its severity, but how can we apply it in clinical practice? We performed the ROC curve on trumpet-model C_{ANO} for screening patients with OSA and nocturnal oxygen desaturation. The threshold of C_{ANO} at 4.5 ppb could identify specifically (94%) patients with high risk of OSA and nocturnal oxygen desaturation. These patients should get priority to polysomnography and potential CPAP treatment.

Our current study presented some limits. We decided to eliminate patients with advanced cardiovascular and/or respiratory disorders, especially those presenting daytime oxygen desaturation and those with heart failure in order to study specifically the effects of OSA on nocturnal hypoxemia and to exclude central sleep apnoea [34]. Patients that could not operate a satisfactory exhaled NO measurements with at least 8-second expiration and 3-second NO plateau levels were also eliminated [23]. As a result, only patients with less severity of OSA were included in this study.

Obesity can induce inflammation and oxidative stress observed in patients with metabolic syndrome and OSA [35]. We found a weak correlation between C_{ANO} and BMI in patients with OSA but in multiple linear regressions, the relationship between C_{ANO} and AHI was independent to BMI. Other studies using the 2-CM model did not find significant correlation between C_{ANO} and BMI, a result that might be accounted for their small populations [10,15].

In conclusion, our study using for the first time the trumpet model to characterise alveolar and bronchial exhaled NO production with correction for axial NO diffusion, demonstrated an increased C_{ANO} in patients with OSA, accentuated after sleep, linking the distal lung inflammation to intermittent hypoxia. We proposed exhaled NO measurement to screen for patients with oxygen desaturation OSA as they presented suggestive symptoms of OSA, especially snoring and daytime sleepiness. Exhaled NO should be further investigated in patients with morbid obesity and metabolic syndrome. Finally, CIH-exposed animal model investigations should be performed to directly study exhaled NO in the relation with NF-kB and inducible NOS pathways to demonstrate precisely what happens in human disease.

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